Ciba Specialty Chemicals Corporation
North America

Consumer Care

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March 29, 1999

OPPT Document Control Officer -TSCA 8(e)
U.S. Environmental Protection Agency
401 M Street, SW
Washington, DC 20460

Via Certified Mail
Return Receipt Requested

Re: Substantial Risk Notification under TSCA § 8(e) for 3-Benzimidazolyl-7-diethylamino-cumarin (CASRN: 27425-55-4)

In accordance with the reporting requirements of TSCA § 8(e), the Colors Division of Ciba Specialty Chemicals Corporation ("Ciba") is hereby providing the U.S. Environmental Protection Agency notification of scientific results indicating previously unobserved skin sensitization effects for the subject chemical. A summary of the study findings are provided below.

In a standard Guinea Pig Maximization test, ten males and ten females were challenged with 0.2 grams of the test substance after the induction treatments. Positive responses occurred in 6 of 20 animals after 24-hours and in 13 of 20 animals after 48-hours. Mild erythema and edema (grade 1) occurred at 24 and 48-hours in 2 of 10 and 1 of 10 control animals, respectively, subjected to the challenge dose. Further adverse effects did not occur during the study. As a result, 3-Benzimidazolyl-7-diethylamino-cumarin (CASRN: 27425-55-4) is considered a skin sensitizer in Guinea Pigs.

Enclosed please find a copy of the laboratory final report [Skin Sensitization Test in the Guinea Pig, Ciba-Geigy Ltd. Test No. 935052, dated October 5, 1993]. Please contact the undersigned if you have any further questions or comments regarding this submission.

Sincerely,

8EHQ-99-14417

Carl D. D'Ruiz, MPH
Executive Director, Product Stewardship and Regulatory Affairs

Attachment

Cc: A. Wiedow
J. Plautz
K. Taft

4090 Premier Drive
P.O. Box 2444
High Point, NC 27261-2444

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Skin Sensitisation Test in the Guinea Pig

Maximisation Test

Test No. 935052

FAT 92279/A

3-Benzimidazolyl-7-diethylamino-cumarin trocken

Report

Study Director: Dr. med.vet. Ch. Hagemann

Testing Facility: CIBA-GEIGY Limited
Toxicology Services
Short-term Toxicology
4332 Stein / Switzerland

Test-Guideline: OECD 406; 92/69/EEC, B.6.

Date of protocol: June 8, 1993

Completion date: October 5, 1993

Sponsor: CIBA-GEIGY Limited
Textile Dyes Division
4002 Basel / Switzerland

This report contains: 30 pages

(14)

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Certification of GLP and verification of the report

(Certification of Good Laboratory Practice and verification of a complete and unaltered copy of the report by the sponsor)

The Statement of Compliance with Good Laboratory Practice found on page 4, and signed by the Study Director is truthful and accurate. This report as provided by the testing facility is complete and unaltered.

For the Sponsor:

Signature: ..... Date: 13.10.93

Statement of compliance with Good Laboratory Practice

This study has been performed in compliance with Good Laboratory Practice (GLP) in Switzerland (Verfahren und Grundsaetze der Guten Laborpraxis (GLP) in der Schweiz), Procedures and Principles, March 1986, issued by the Swiss Federal Department of the Interior and the Intercantonal Office for the Control of Medicaments. These procedures are in essence consistent with:

- OECD Principles of Good Laboratory Practice (Council Decision 81/30, adopted on May 12, 1981, and the OECD Recommendation 83/95 concerning the 'Mutual Recognition of Compliance with Good Laboratory Practice', adopted on July 26, 1983).
- United States Environmental Protection Agency, Title 40 Code of Federal Regulations Part 160 (FIFRA); Federal Register, August 17, 1989.
- United States Environmental Protection Agency, Title 40 Code of Federal Regulations Part 792 (TSCA); Federal Register, August 17, 1989.
- Japan Ministry of Agriculture, Forestry and Fisheries, NohSan, Notification No. 3850, Agricultural Production Bureau, August 10, 1984.

Study director: Dr. med.vet. Ch. Hagemann

Signature: Ch. Hagemann ..... Date: October 5, 1993

Signatures

This report represents the results of the investigations compiled by the undersigned:

Study director: Dr. med.vet. Ch. Hagemann

Signature: ..... Date: October 5, 1993

Reserved page for flagging statements

Quality assurance statement

Test Article:	**FAT 92279/A**
Study Title:	**Skin Sensitisation Test in the Guinea Pig**
Test Number:	**935052**
Study Director:	**Dr. med.vet. Ch. Hagemann**

I hereby certify that the following Quality Assurance activities were performed:

QA-Activity	Date performed	Date reported
Facility Inspection	17.03.93	02.04.93
Protocol Audit	10.06.93	10.06.93
Final Report Audit	23.09.93	27.09.93

Quality Assurance Inspector: for D. Baltisberger (absent)
W.W. Hartmann

Signature: W W Hartmann ..... Date: October 6 1993

1. SUMMARY AND CONCLUSION

30 and 65% of the animals of the test group were sensitised by FAT 92279/A under the experimental conditions employed.

According to the maximisation grading FAT 92279/A showed a strong grade of skin-sensitising (contact allergenic) potential in albino guinea pigs.

2. GENERAL

2.1. Introduction

At the request of the Textile Dyes Division of CIBA-GEIGY Limited, a sensitisation test in albino guinea pigs was performed to determine the contact allergenic potency of FAT 92279/A in albino guinea pigs.

This test was based on the OECD Guideline No. 406, adopted May 12, 1981, adapted July 17, 1992, by the OECD council, and on Annex V, Part B of Council Directive 67/548/EEC (Commission Directive 92/69/EEC of July 31, 1992).

Experimental starting date:	June 21, 1993
Experimental termination date:	July 22, 1993
Testing facility:	CIBA-GEIGY Limited Toxicology Services Short-term Toxicology 4332 Stein/Switzerland

2.2. Archives

Archives are located at CIBA-GEIGY Limited, Werk Stein, CH-4332 Stein, Switzerland. Raw data, protocol and report will be stored at this location.

2.3. Distribution

Sponsor (Dr. J. Maldacker-Kurth)
Archives

2.4. Test material

Test article:	FAT 92279/A
Batch No.:	210602.92
Additional specification:	3-Benzimidazolyl-7-diethylamino-cumarin trocken
Contents/Purity:	99.9%
Physical properties:	solid; yellow to orange powder
Storage conditions:	room temperature
Validity:	February, 1994
Test article received:	May 25, 1993

2.5. Auxiliary compounds

- Physiological saline (0.9 %), sterile solution (Hausmann, St. Gallen, Switzerland)
- Bacto Adjuvant, Complete, Freund (Difco Lab. Detroit, Michigan USA)
- Vaseline (white petrolatum) Ph. H. VI (Siegfried AG, Zofingen, Switzerland)
- Oleum arachidis Ph. H. VI (Siegfried AG, Zofingen, Switzerland

2.6. Test System

The albino guinea pig is the recommended species for skin sensitisation studies.

Animal strain:	Pirbright White Strain (Tif: DHP)
Breeder:	CIBA-GEIGY Limited Animal Production 4332 Stein / Switzerland
Date of acclimatisation:	June 16, 1993

2.7. Group Size and Husbandry

The test was performed on 10 male and 10 female guinea pigs in the test group and 5 males and 5 females in the control group, respectively, initially weighing between 324 to 407 g.

The animals were housed individually in Macrolon cages (Type 3), assigned to the different groups by means of random numbers generated by the random number generator, identified by individual ear tags, kept at a constant room temperature of $22\pm3^{\circ}C$, at a relative humidity of 30 to 70% and a 12 hours light cycle day.

The animals received ad libitum standard guinea pig pellets - NAFAG No. 845, Gossau SG and fresh water.

All batches of the diet are assayed for nutritive ingredients and contamination level by the manufacturer. Analytical results are available at the animal supply office.

The drinking water quality fulfilled the critical parameters in the specifications of the "Schweizerisches Lebensmittelbuch" (Edition 1972). The results of the routine chemical examination of water at source (Grundwasserfassung Stein) as conducted periodically by the water authority (Baudepartement des Kantons Aargau, Abteilung Gewaesserschutz) are available to CIBA-GEIGY Limited, as well as the results of inhouse chemical analysis by the analytical laboratories of the Pharmaceutical Division, CIBA-GEIGY Limited.

2.8. Sensitivity of strain

The sensitivity of the strain is checked once or twice a year with a known mild to moderate sensitiser, such as mercaptobenzothiazole, hexyl cinnamic aldehyde or potassiumdichromate.

The results of the latest positive control test are presented in Appendix 3 of this report.

3. METHODS

3.1. Reason for selection

The maximisation test has been selected, because it is one of the recommended tests in the OECD guideline 406, adopted May 12, 1981, adapted July 17, 1992, as well as in Annex V, Part B of Council Directive 67/548/EEC (Commission Directive 92/69/EEC of July 31, 1992), and because the sensitivity of the method is well known. The test has been performed according to the original protocol of Magnusson and Kligman (J. invest. Dermatol. 52, 268-276, 1969; Contact Dermatitis 6, 46-50, 1980).

3.2. Test procedure and concentrations used

3.2.1. General

A test group of 20 animals (10 m/10 f) and a control group of 10 animals (5 m/5 f) were used.

3.2.2. Induction procedure

The induction was a two-stage operation. First, intradermal injections (into the neck region); second, closed patch exposure over the injection sites one week later.

First induction week, intradermal injection

Three and two pairs of intradermal injections (0.1 ml per injection) were made simultaneously into the shaved neck of the guinea pigs of the test and control group, respectively.

Test group:

- adjuvant/saline mixture 1:1 (v/v)
- 5% FAT 92279/A in Oleum arachidis (w/v)
- 5% FAT 92279/A in the adjuvant/saline mixture (w/v)

Control group:

- adjuvant/saline mixture 1:1 (v/v)
- Oleum arachidis

Second induction week, epidermal application

In the test group FAT 92279/A was incorporated in vaseline (w/w) and applied on a filterpaper patch to the neck of the animals (patch 2x4 cm; approx. 0.4 g per patch; occluded administration for 48 hours). The control group was treated with the vehicle only.

Test group:

- 50% FAT 92279/A in vaseline

Control group:

- vaseline only

3.2.3. Rest period

During weeks 3 and 4 no treatments were performed.

3.2.4. Challenge (week 5)

The test and control group animals were tested on the flank with FAT 92279/A in vaseline (w/w) and the vehicle alone (patch 2x2 cm; approx. 0.2 g per patch; occluded administration for 24 hours).

Test and control group:

- 50% FAT 92279/A in vaseline

3.2.5. Pretests

Intradermal Induction

The concentration for the intradermal injections was selected on account of the solubility of the test article in standard vehicles and its local and systemic tolerability in a pretest. The following concentration of test article has been prepared for intradermal injection:

5% in Oleum arachidis.

Since 5% FAT 92279/A in Oleum arachidis could be injected and was well tolerated, this concentration was used for the intradermal induction.

Epidermal Applications (induction and challenge)

The concentrations for the epidermal applications were selected on account of the primary irritation potential of the test article. The following concentrations of FAT 92279/A have been examined on separate animals for the determination of the maximum subirritant concentration (see also Table 4):
30 and 50% in vaseline.
50% in vaseline was the highest applicable concentration of the test article.
The tested concentrations did not induce erythema reactions.

Therefore the application site was pretreated with 10% sodium-laurylsulfate (open application) 24 hours prior to the epidermal induction application.

3.3. Observations and records

Induction reactions

After the intradermal and the epidermal induction application irritant reactions are normally induced by the adjuvant, the high test article concentration, or the sodium lauryl sulfate pretreatment. Because most of the reactions are treatment related and not compound related, the reactions are only described in special cases in the section of results.

Challenge reactions

Twenty four and forty eight hours after removing the dressings, the challenge reactions were graded according to the Draize scoring scale (Appendix 1).

General

The body weight was recorded at start and end of the test.

3.4. Interpretation of results

The sensitising potential of FAT 92279/A was classified according to the grading of Magnusson and Kligman (Appendix 2).

According to the guide to the labelling of dangerous substances and the criteria for the choice of sentences indicating particular hazards (R sentences) attributed to dangerous substances (Commission Directive 93/21/EEC, April 27, 1993) a test article was classified as a sensitiser in the case where a positive response was noted in at least 30 % of the animals.

4. RESULTS

The incidence of positive animals per group, the individual challenge reactions and the evaluation of the primary skin irritation potential are listed in Tables 1, 2, 3 and 4.

The individual animal weights at start and end of the test are listed in Table 5.

Under the experimental conditions employed, 30 and 65% of the test group animals and 20 and 10% of the control group animals showed skin reactions 24 and 48 hours after removing the dressings, respectively. Because of the rather large difference between test and control group at the 48 hours evaluation (65 and 10%, respectively) a rechallenge was not considered necessary.

FAT 92279/A is, therefore, classified as a strong sensitiser in albino guinea pigs according to the grading of Magnusson and Kligman.

According to the EEC classification criteria (Commission Directive 93/21/EEC, April 27, 1993) FAT 92279/A did show a skin-sensitising (contact allergenic) potential in albino guinea pigs.

5. TABLES

TABLE 1

Number of positive animals per group after occlusive epidermal application

Control group:

	after 24 hours	after 48 hours
vehicle control	0/10	0/10
test article	2/10	1/10

Test group:

	after 24 hours	after 48 hours
vehicle control	0/20	0/20
test article	6/20	13/20

TABLE 2

Challenge reactions after epidermal application (CONTROL GROUP)

DRAIZE Score 24 hours after removal of the dressing

Vehicle control

Male animals	181	182	183	184	185
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

Female animals	196	197	198	199	200
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

Test article control

Male animals	181	182	183	184	185
Erythema score:	0	0	1	1	0
Edema score:	0	0	0	1	0

Female animals	196	197	198	199	200
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

DRAIZE Score 48 hours after removal of the dressing

Vehicle control

Male animals	181	182	183	184	185
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

Female animals	196	197	198	199	200
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

Test article control

Male animals	181	182	183	184	185
Erythema score:	0	0	0	1	0
Edema score:	0	0	0	1	0

Female animals	196	197	198	199	200
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

TABLE 3

Challenge reactions after epidermal application (TEST GROUP)

DRAIZE Score 24 hours after removal of the dressing

Vehicle control

Male animals	186	187	188	189	190	191	192	193	194	195
Erythema score:	0	0	0	0	0	0	0	0	0	0
Edema score:	0	0	0	0	0	0	0	0	0	0

Female animals	201	202	203	204	205	206	207	208	209	210
Erythema score:	0	0	0	0	0	0	0	0	0	0
Edema score:	0	0	0	0	0	0	0	0	0	0

Test article

Male animals	186	187	188	189	190	191	192	193	194	195
Erythema score:	0	1	0	1	0	0	0	0	0	1
Edema score:	0	0	0	1	0	0	0	0	0	1

Female animals	201	202	203	204	205	206	207	208	209	210
Erythema score:	0	0	1	0	0	0	1	1	0	0
Edema score:	0	0	0	0	0	0	1	0	0	0

DRAIZE Score 48 hours after removal of the dressing

Vehicle control

Male animals	186	187	188	189	190	191	192	193	194	195
Erythema score:	0	0	0	0	0	0	0	0	0	0
Edema score:	0	0	0	0	0	0	0	0	0	0

Female animals	201	202	203	204	205	206	207	208	209	210
Erythema score:	0	0	0	0	0	0	0	0	0	0
Edema score:	0	0	0	0	0	0	0	0	0	0

Test article

Male animals	186	187	188	189	190	191	192	193	194	195
Erythema score:	0	1	1	1	1	0	1	1	1	1
Edema score:	0	0	0	1	0	0	0	0	0	1

Female animals	201	202	203	204	205	206	207	208	209	210
Erythema score:	0	0	1	1	0	0	1	1	0	1
Edema score:	0	0	0	0	0	0	0	0	0	0

TABLE 4

Evaluation of the primary skin irritation potential

Procedure: On each animal 2 concentrations of FAT 92279/A were applied simultaneously on the left and right flank. A naive skin site served as control (not reported).
7 days before application of FAT 92279/A two pairs of intradermal injections of an adjuvant/saline mixture 1:1 (v/v; 0.1 ml per injection) were made simultaneously into the shaved neck of the guinea pigs.

	score 24 hours after removing the dressing		score 48 hours	
	concentrations of FAT 92279/A in vaseline (w/v;%)			
Animal No. / sex	30 % er/ed	50 % er/ed	30 % er/ed	50 % er/ed
1 male	0/0	0/0	0/0	0/0
2 female	0/0	0/0	0/0	0/0

ed = edema, er = erythema

TABLE 5

Individual animal bodyweights in g - males

CONTROL GROUP Animal No.	weight at start	weight at end	TEST GROUP Animal No.	weight at start	weight at end
181	366	542	186	381	524
182	357	535	187	365	523
183	392	560	188	372	516
184	362	488	189	377	562
185	364	513	190	381	574
			191	382	501
			192	369	533
			193	333	507
			194	396	540
			195	364	526
Mean	368	528		372	531
Std.Dev.	13.7	27.8		16.7	22.9

Individual animal bodyweights in g - females

CONTROL GROUP Animal No.	weight at start	weight at end	TEST GROUP Animal No.	weight at start	weight at end
196	380	489	201	351	484
197	349	469	202	374	519
198	324	418	203	388	484
199	348	503	204	407	552
200	355	476	205	373	469
			206	356	527
			207	349	486
			208	344	527
			209	333	468
			210	365	498
Mean	351	471		364	501
Std.Dev.	20.0	32.3		22.2	28.3

6. APPENDICES

APPENDIX 1

Evaluation of skin reactions

Evaluation of skin reactions according to Draize in Appraisal of the Safety of chemicals in Foods, Drugs and Cosmetics (1959), The US Association of Food and Drug Officials (AFDO).

Erythema and eschar formation

No erythema.....0
Very slight erythema (barely perceptible).....1
Well defined erythema.....2
Moderate to severe erythema.....3
Severe erythema (beet redness) to slight eschar formation (injuries in depth).....4

Edema formation

No edema.....0
Very slight edema (barely perceptible).....1
Slight edema (edges of area well defined by definite raising).....2
Moderate edema (raised approximately 1 mm).....3
Severe edema (raised more than 1 mm and extending beyond area of exposure).....4

APPENDIX 2

Maximisation grading

Sensitisation rate (%)	Grade	Classification
0 - 8	I	weak
9 - 28	II	mild
29 - 64	III	moderate
65 - 80	IV	strong
81 - 100	V	extreme

APPENDIX 3

Reference values with Potassiumdichromate

Test No. 920024

Experimental starting date: November 11, 1992
Experimental completion date: December 11, 1992

The following concentrations of the reference compound and vehicles were used:

Intradermal induction

Concentration of compound: 0.2%
Vehicle: physiological saline

Epidermal induction

Concentration of compound: 5%
Vehicle: vaseline

Epidermal challenge

Concentration of compound: 1%
Vehicle: vaseline

Number of positive animals per group after occlusive epidermal application

Control group:

	after 24 hours	after 48 hours
vehicle control	0/10	0/10
test article	0/10	0/10

Test group:

	after 24 hours	after 48 hours
vehicle control	0/10	0/10
test article	9/10	9/10

Challenge reactions after epidermal application (CONTROL GROUP)

DRAIZE Score 24 hours after removal of the dressing

Vehicle control

Male animals	141	142	143	144	145
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

Female animals	146	147	148	149	150
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

Test article control

Male animals	141	142	143	144	145
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

Female animals	146	147	148	149	150
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

DRAIZE Score 48 hours after removal of the dressing

Vehicle control

Male animals	141	142	143	144	145
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

Female animals	146	147	148	149	150
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

Test article control

Male animals	141	142	143	144	145
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

Female animals	146	147	148	149	150
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

DRAIZE Score 48 hours after removal of the dressing

Vehicle control

Male animals	131	132	133	134	135
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

Female animals	136	137	138	139	140
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

Test article

Male animals	131	132	133	134	135
Erythema score:	0	2	1	1	2
Edema score:	0	1	0	0	0

Female animals	136	137	138	139	140
Erythema score:	1	1	2	1	2
Edema score:	0	0	1	0	0

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